

Amendments to the Specification

Please replace paragraphs 0007, 0008, 0011-0014, 0016, 0018, 0035, 0040, 0041, 0043, 0049 and 0064 with the following paragraphs:

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[0007] Another aspect of the present invention provides methods for using the nucleic acid of SEQ ID NO:1 ~~or SEQ ID NO:3~~, which encodes the amino acid sequence of a mutant pyruvate carboxylase. Such uses include the replacement of the wild-type pyruvate carboxylase with the feedback-resistant pyruvate carboxylase, and the production of amino acids. An additional aspect of the present invention provides a polypeptide comprising the amino acid sequence of SEQ ID NO:2 ~~or SEQ ID NO:4~~. Still another aspect of the present invention provides a polypeptide comprising the amino acid sequence selected from the group comprising SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:18.

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[0008] Another aspect of the present invention also relates to a nucleic acid molecule comprising a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:2, ~~SEQ ID NO:4~~, or the amino acid sequence encoded by the DNA contained in Deposit Number NRRL B-11474. Another aspect of the present invention further relates to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 ~~and SEQ ID NO:3~~.

[0011] ~~Figures 3A-3B show the full length nucleotide sequence (SEQ ID NO:3)~~

~~encoding the amino acid sequence of feedback resistant pyruvate carboxylase.~~

[0012] ~~Figure 4~~ Figure 3 shows the effects of various substrate concentrations on the pyruvate carboxylase activity in *C. glutamicum* ATCC 21253 and NRRL B-11474.

B³ [0013] ~~Figure 5~~ Figure 4 shows the effects of aspartate concentration on the activity of pyruvate carboxylase in *C. glutamicum* ATCC 21253 and NRRL B-11474.

[0014] ~~Figure 6~~ Figure 5 shows the effects of acetyl-CoA concentration on the activity of pyruvate carboxylase in *C. glutamicum* ATCC 21253 and NRRL B-11474.

[0016] An embodiment of the present invention relates to an isolated or purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

B⁴ (a) a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:2, ~~(b) a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:4,~~ (c) (b) a nucleotide sequence encoding the amino acid sequence encoded by the DNA contained in Deposit Number NRRL B-11474 or ~~(d) (c)~~ a nucleotide sequence complementary to any of the nucleotide sequences in ~~(a), (b), or (e)~~ (a) or (b).

B⁵ [0018] Another aspect of the invention is directed to nucleic acid molecules at least 90%, 95%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), ~~Figure 3 (SEQ ID NO:3)~~ or to the nucleic acid sequence of the deposited DNA

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(NRRL B-30293, deposited May 30, 2000).

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[0035] The present invention provides methods for utilizing the nucleic acid of SEQ ID NO:1 ~~or SEQ ID NO:3~~, which encodes the amino acid sequence of a mutant pyruvate carboxylase. Such methods include the replacement of the wild-type pyruvate carboxylase with the feedback-resistant pyruvate carboxylase, and the production of amino acids. The method for replacement of a wild-type pyruvate carboxylase gene, with a feedback resistant pyruvate carboxylase gene, in a *Corynebacterium glutamicum* host cell comprises the steps of: (a) replacing a genomic copy of the wild-type pyruvate carboxylase gene with a selectable marker gene through homologous recombination to form a first recombinant strain; and (b) replacing the selectable marker gene of step (a) in the first recombinant strain, with the feedback resistant pyruvate carboxylase gene through homologous recombination to form a second recombinant strain. The homologous recombination in steps (a) and (b) would occur between the genetic material of the host cell and any of the vectors of the present invention.

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[0040] The present invention provides an isolated or purified polypeptide encoded by the DNA plasmid encoding pyruvate carboxylase contained in Deposit Number NRRL B-30293, or the amino acid sequence of SEQ ID NO:2 ~~or the amino acid sequence of SEQ ID NO:4~~. Still another aspect of the present invention provides a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:18.

[0041] Accordingly, SEQ ID NO:6 corresponds to the amino acid sequence:
PSKNIDDIVKSAE. SEQ IN NO:8 corresponds to the amino acid sequence:
RGMRFVSSPDELK. SEQ ID NO:10 corresponds to the amino acid sequence:
B⁸ AAFGDGSVYVEFA. SEQ ID NO:12 corresponds to the amino acid sequence:
VQILGDRTGEVVH. SEQ ID NO:14 corresponds to the amino acid sequence:
IATGFIGDHPHLL. SEQ ID NO:16 corresponds to the amino acid sequence:
TITASVEGKIDRV. SEQ ID NO:18 corresponds to the amino acid sequence:
MTAITLGGLLLKGITLV MTAITLGGLLLKGITLV.

B⁹ [0043] One aspect of the present invention include the polypeptides which are at least
80% identical, more preferably at least 90%, 95% or 100% identical to the polypeptide
encoded by the DNA plasmid encoding pyruvate carboxylase contained in Deposit
Number NRRL B-30293, the polypeptide of SEQ ID NO:2 ~~or the polypeptide of SEQ ID~~
NO:4.

B¹⁰ [0049] As a practical matter, whether any particular polypeptide is, for instance, 95%
identical to the amino acid sequence shown in SEQ ID NO:2, ~~SEQ ID NO:4~~ or to the
amino acid sequence encoded by deposited DNA clone can be determined conventionally
using known computer programs such the Bestfit program (Wisconsin Sequence
Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research
Park, 575 Science Drive, Madison, Wis. 53711). When using Bestfit or any other

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sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

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[0064] Pyruvate carboxylase activity was determined as a function of various concentrations of its substrates: pyruvate, bicarbonate and ATP (~~Figure 4~~) (Figure 3). Based on the data generated, the affinity constants of pyruvate carboxylase for its substrates were determined (Table 2). The pyruvate carboxylase from NRRL B-11474 (also known as BF100) and ATCC 21253 strains demonstrated a similar affinity for pyruvate and ATP. Pyruvate carboxylase activity in both strains were inhibited by ATP above a concentration of 2 mM. However pyruvate carboxylase in ATCC 21253 had a higher affinity for bicarbonate than pyruvate carboxylase from NRRL B-11474 (BF100).

Please replace Table 2 on page 22 with the following Table 2:

B12

Strain	$K_m(\text{pyruvate})[\text{mM}]$	$K_m(\text{HCO}_3^-)[\text{mM}]$	$K_M(\text{ATP})[\text{mM}]$
<i>C. glutamicum</i>			
Pyc BF100	1.3 ± 0.3 <u>0.3</u>	14.4 ± 4	0.4 ± 0.1
NRRL B-11474			
Pyc ATCC 21253	0.3 ± 0.1	2.9 ± 0.8	0.3 ± 0.1

Please add the following paragraph to page 24, after paragraph [0067]:

B13

NRRL B-30293 was deposited on May 30, 2000 at the Agricultural Research Culture Collection (NRRL), International Depository Authority; 1815 North University

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Street; Peoria, Illinois, 61064 U.S.A. All strains were deposited under the terms of the Budapest Treaty.

Please replace the previous sequence listing with the present sequence listing, at the end of the application.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above-mentioned application are the same.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.